

WHAT IS CLAIMED IS:

1. A method for determining the antigen specific activation of T cells from a human or animal subject comprising:

(i) culturing a sample of whole blood of said human or animal with an antigen specific stimulus; and

(ii) analyzing an aliquot of said cultured sample for expression of one or more intracellular cytokines and/or one or more early activation antigens in combination with one or more T cell markers, by means of multi-parameter flow cytometry.

2. The method of Claim 1 further comprising lysing the red blood cells and washing to remove the debris prior to the measuring step.

3. The method of Claim 1 further comprising providing exogenous costimulation of said cells by adding a costimulus to said sample contemporaneous with the addition of said antigen specific stimulus.

4. The method of Claim 3 wherein the costimulus is selected from the group consisting of ligands or antibodies to CD28, CD40, CD86, and CD118.

5. The method of Claim 1 wherein the antigen specific stimulus is selected from the group consisting of alloantigens, viral antigens, autoantigens, peptides, and proteins.

6. The method of Claim 5 wherein the antigen specific stimulus is selected from the group consisting of cytomegalovirus antigen, measles virus antigen, Mycobacterium tuberculosis antigen, and mumps virus antigen.

7. The method of Claim 1 wherein said intracellular cytokines are selected from the group consisting of γ -IFN, IL-2, IL-4, and TNF- α .

8. The method of Claim 1 wherein early activation antigen is CD69.

9. The method of Claim 1 wherein the sample is cultured with the antigen specific stimulus for a period of 24 hours or less.

10. The method of Claim 1 wherein the sample is cultured with the antigen specific stimulus for a period of 6 hours or less.

11. The method of Claim 1 further comprising culturing the sample with an immunosuppressive or immuno augmenting drug, simultaneous with the culturing with the antigen specific stimulus.

12. The method of Claim 1 wherein the sample of whole blood is obtained from a human or animal treated with an immunosuppressive or an immunoaugmenting drug.

13. The method of Claim 1 further comprising adding a reagent which blocks secretion of intracellular cytokines during the culturing of the sample with the antigen specific stimulus.

14. The method of Claim 13 wherein the reagent is Brefeldin A.

15. The method of Claim 13 wherein the reagent is added after the culturing has proceeded for at least 1 hour.

16. The method of Claim 1 further comprising adding a cationic chelating agent after the culturing is complete but prior to the measuring.

17. The method of Claim 16 wherein the cationic chelating agent is EDTA or EGTA.

18. The method of Claim 1 wherein the lysing of the red blood cells is accomplished using a lysing agent.

add
a
1H₂O
B₃